



UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

KELLY FERREIRA DOS SANTOS

**IMPACTO DA INGESTÃO DE PROTEÍNAS DO SORO DO LEITE
PRÉ-HIDROLISADAS NA DIABETES MELITUS INDUZIDA EM
RATOS**

***IMPACT OF PRE-HYDROLYZED MILK WHEY PROTEIN INTAKE
ON INDUCED DIABETES MELLITUS IN RATS***

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição, da Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas para obtenção do título de Mestra em Alimentos e Nutrição na área de concentração: Nutrição Experimental Aplicada à Tecnologia de Alimentos

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Orientador: Prof. Dr. Jaime Amaya-Farfán

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RESUMO GERAL

O presente trabalho teve por objetivo avaliar a capacidade da proteína do soro do leite, sob suas duas formas (concentrada e hidrolisada) em auxiliar na prevenção do desenvolvimento de obesidade e diabetes em ratos Wistar diabéticos. Foram utilizados 40 ratos machos Wistar de 8 semanas, alimentados com dieta hiperlipídica por 31 dias. No 20º dia de dieta, os animais receberam uma dosagem de 40mg/kg de estreptozotocina (STZ). Os grupos experimentais eram: CASControl – animais alimentados com dieta normolipídica AIN-93G, contendo caseína como fonte proteica; HFCAS – dieta hiperlipídica, com 62% do valor calórico total (VCT) em lipídios, e caseína como fonte proteica; HFPSL - dieta hiperlipídica, com 62% do VCT em lipídios e concentrado de proteína do soro do leite como fonte proteica; HFPSLH – dieta hiperlipídica, com 62% do VCT em lipídios e proteína do soro de leite hidrolisada como fonte proteica. Foi realizado teste de tolerância à glicose (GTT) e, ao término do experimento, as dosagens séricas de glicose, aspartato aminotransferase (AST), alanina aminotransferase ALT, albumina, proteínas totais, colesterol total, HDL-c, triacilgliceróis e insulina, além de análise da composição centesimal das carcaças liofilizadas e fígado dos animais. O grupo HFPSLH apresentou menor ganho ponderal em relação aos demais grupos tratados com dieta hiperlipídica, além de tendência a uma melhor reação glicêmica no GTT. Após administração de STZ, todos os grupos com dieta hiperlipídica desenvolveram hiperglicemia e alguns danos hepáticos. Os dados sugerem que substituição da caseína pela proteína do soro do leite hidrolisada foi significativamente positiva no sentido de amenizar o excessivo ganho de peso e o nível hiperglicêmico produzidos pelo tratamento. Por outro lado, o parâmetro clássico da função hepática, ALT, e os níveis de triacilgliceróis séricos sugeriram a existência de um possível antagonismo entre o uso da hepatotóxica estreptozotocina e as proteínas do soro lácteo.

IMPACT OF PRE-HYDROLYZED MILK WHEY PROTEIN INTAKE ON INDUCED DIABETES MELLITUS IN RATS

ABSTRACT

The purpose of this study was to explore the possible ability of the milk whey protein, under its two forms (concentrated and hydrolyzed) to assist in preventing the development of obesity and diabetes in rats submitted to a diabetes-induction procedure. Forty 8-week old male Wistar rats were fed a high fat (HF) diet for 31 days and, on day 20, received an injection of 40mg/kg streptozotocine (STZ). The experimental groups were: CAS Control (animals fed the AIN 93-G normolipidic diet, containing casein as protein source); HFCAS (HF diet, with 62% TCV in lipids plus casein as protein source); HFWPC (HF diet, with 62% TCV in lipids plus whey-protein concentrate (WPC) as protein source); HFWPH (HF diet, with 62% TCV in lipid plus whey-protein hydrolyzate (WPH) as a protein source). At the end of the feeding experiment, the following tests were performed: glucose tolerance test (GTT), serum glucose, AST, ALT, albumin, total protein, total cholesterol, HDL-c, triacylglycerols and insulin, as well as analysis of the chemical composition of lyophilized carcasses and livers of the animals. The HFWPH group exhibited lower weight gain than the other groups treated with HF diet, and a tendency to a better glycemic response in the GTT. After administration of the STZ, all groups developed the expected hyperglycemia, body-fat accumulation and some liver damage. The data suggest that replacement of casein by the whey-protein hydrolyzate was significantly positive in order to mitigate excessive weight gain and the hyperglycemia produced by the treatment. On the other hand, the classic parameter of liver function, ALT and serum triacylglycerols suggested the existence of a possible antagonism between the use of hepatotoxic streptozotocin and whey proteins.

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LISTA DE SIGLAS

AGRP – Peptídeo relacionado ao Agout

AIN-93G – Dieta composta por 20% de proteína, elaborada com teores vitamínicos e minerais capazes de promover o crescimento e desenvolvimento saudáveis de roedores (Reeves *et al.*, 1993)

ALT – Alanina aminotransferase

AST – Aspartato aminotransferase

AUC – Área sob a curva

BCAAs – Aminoácidos de cadeia ramificada

CCEA-IB – Comissão de ética na experimentação animal da Universidade Estadual de Campinas

CCK – Colecistoquinina

CEMIB – Centro multidisciplinar para investigação biológica

DM2 – Diabetes mellitus tipo 2

DPP-IV – Enzima dipeptidyl peptidase IV

GLP-1 – Peptídeo semelhante ao glucagon

GTT – Teste de tolerância à glicose

HDL – Lipoproteína de alta densidade

HFCAS – Dieta ou grupo de ratos alimentados com dieta hiperlipídica com caseína como fonte proteica e administração de STZ 40mg/kg de peso corporal

HFWPC – Dieta ou grupo de ratos alimentados com dieta hiperlipídica com proteína do soro do leite concentrada como fonte proteica e administração de STZ 40mg/kg de peso corporal

HFWPH – Dieta ou grupo de ratos alimentados com dieta hiperlipídica com proteína do soro do leite hidrolisada como fonte proteica e administração de STZ 40mg/kg de peso corporal

IRA – Radioimunoensaio

LDL-c – colesterol em lipoproteína de baixa densidade

LEB – Laboratório de ensaios biológicos

CAS CONTROL – ratos Wistar tratados com dieta padrão e injeção de tampão citrato

PSL – Proteína do soro do leite

SPF – Animais livres de patógenos específicos

STZ – Estreptozotocina

TAG – Triacilgliceróis

WP – Whey protein

WPC – Whey protein concentrate

WPH – Whey protein hydrolyzate

INTRODUÇÃO GERAL

A prevalência mundial de diabetes é crescente. Segundo dados da International Diabetes Federation (2012), mais de 371 milhões de pessoas são portadores de diabetes no mundo. Os países que lideram o *ranking* são China, Índia, Estados Unidos e Brasil. Além disso, há uma alta mortalidade causada por complicações da doença, que apenas em 2012 causou cerca de 4,8 milhões de óbitos.

A descoberta de alimentos e ingredientes funcionais que possam contribuir para a prevenção da hiperglicemia ou controle dos níveis glicêmicos e aumento da sensibilidade à insulina se torna importante, levando em conta o alto grau de prevalência mundial de diabetes tipo 2 (DM2).

Estudos de nosso grupo de pesquisa têm evidenciado diversos benefícios atribuídos ao consumo das proteínas do soro do leite. Além de auxiliar na melhora do desempenho muscular de atletas (LOLLO *et al.*, 2011; TASSI *et al.*, 1998; PIMENTA *et al.*, 2006; NERY-DIEZ *et al.*, 2010; MORATO *et al.*, 2013; MOURA *et al.*, 2013), ensaios recentes demonstram que a proteína do soro do leite hidrolisada, bem como os dipeptídeos, formados durante o processo de digestão destas proteínas, promove um aumento na captação de glicose, independente de insulina em ratos não obesos sedentários e exercitados (MORATO *et al.*, 2013 a,b).

A autora optou por apresentar esta Dissertação em dois capítulos: o Capítulo 1 faz uma abordagem teórica das proteínas do soro de leite e como sua ingestão poderia produzir benefícios à saúde; o Capítulo 2 discorre sobre o trabalho experimental com o objetivo de avaliar o efeito da ingestão das proteínas do soro de leite sobre o controle glicêmico e a resposta insulínica de ratos Wistar, os quais foram submetidos a uma dieta hiperlipídica, associada à aplicação do antibiótico estreptozotocina, com a finalidade de induzir um quadro de diabetes mellitus.

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CAPÍTULO 1
REVISÃO DE LITERATURA

**BENEFÍCIOS DO CONSUMO DAS PROTEÍNAS DO SORO DO LEITE NA
MITIGAÇÃO DE DOENÇAS CRÔNICAS NÃO TRANSMISSÍVEIS**

CAPÍTULO 1
REVISÃO DE LITERATURA
BENEFÍCIOS DO CONSUMO DAS PROTEÍNAS DO SORO DO LEITE NA
MITIGAÇÃO DE DOENÇAS CRÔNICAS NÃO TRANSMISSÍVEIS

1. Introdução

O soro do leite é extraído durante o processo de coagulação, enzimática ou ácida, da caseína para fabricação do queijo. Corresponde a aproximadamente 20% do total de proteínas do leite, sendo composto principalmente pelas proteínas β -lactoglobulina, α -lactalbumina, albumina de soro bovino, lactoferrina, imunoglobulinas, o dissacarídeo lactose, e minerais solúveis (MARSHALL, 2004).

As proteínas presentes no soro do leite contêm altas concentrações de aminoácidos de cadeia ramificada (BCAAs), como leucina, isoleucina e valina, importantes para construção, manutenção e reparação muscular e também possuem um razoável conteúdo de aminoácidos sulfurados (metionina e cisteína) (BOWEN *et al.*, 2006).

Elas são facilmente digeríveis e rapidamente absorvidas, aumentando mais rapidamente a aminoacidemia após sua ingestão, em comparação com a caseína, facilitando assim a recuperação e manutenção proteicas e o favorecimento da homeostase proteica (BOIRIE *et al.*, 1997).

Nas últimas décadas, diversas pesquisas têm evidenciado a qualidade nutricional e as propriedades funcionais das proteínas do soro do leite, não apenas com fins na nutrição esportiva, mas também na sua utilidade na área médica como tratamento auxiliar no combate e prevenção de doenças tais como a obesidade e a diabetes (JAKUBOWICZ & FROY., 2012; SOUSA *et al.*, 2012).

2. As proteínas do soro do leite

2.1 Características composicionais próprias e em relação às caseínas

A proteína do soro do leite é um subproduto da fabricação de queijos, que era considerado pela indústria um produto residual. Após a descoberta de suas propriedades funcionais, tem sido objeto de estudo em várias áreas do conhecimento.

O leite contém caseína (80%) e proteínas do soro (20%), duas fontes primárias de proteína. No processo de obtenção do queijo, a caseína é coagulada enquanto a proteína do soro do leite se mantém na fase aquosa remanescente.

As proteínas do soro do leite não são apenas boas fontes de aminoácidos, mas também fonte de peptídeos bioativos gerados durante o processo de digestão. Estes peptídeos ligam-se a receptores no lúmen intestinal antes da absorção ou em receptores de órgãos-alvo após absorção para a corrente sanguínea (LUHOVYY *et al.*, 2007).

2.2 Concentrados proteicos do soro do leite

O concentrado proteico do soro do leite é produzido por um processo de clarificação, ultrafiltração, filtração e secagem. O teor proteico do concentrado pode variar entre 35 a 80%. É utilizado na indústria de alimentos devido a diversas propriedades atribuídas às suas proteínas, como sua solubilidade em água, propriedade geleificante, capacidade de transportar diversas moléculas lipofílicas e possuir ação tensoativas (OHATA *et al.*, 2005), sendo empregada em produtos como fórmulas infantis e suplementos para atletas e diversas situações clínicas (SILVA, 2009).

2.3 As proteínas isoladas do soro do leite

Os isolados da proteína do soro do leite são produzidos em menor escala em relação aos concentrados para atender as demandas de alto teor proteico dos produtos finais que requerem a adição de outros ingredientes volumosos. Apesar da matéria-prima do isolado e do concentrado ser o soro de

leite, as composições de proteínas e aminoácidos destes dois compostos são diferentes. Durante seu processo de purificação, o soro do queijo perde o p-kappa-caseína solúvel e alguns aminoácidos, dando origem ao isolado. Após a secagem, o teor de proteínas pode chegar a 90%.

2.4 As proteínas hidrolisadas do soro do leite

Consiste na proteína do soro do leite concentrada que passa por um processo de hidrólise, e tem ampla aplicação na indústria de alimentos por possuir taxa de absorção intestinal 10 vezes maior que aminoácidos livres, além de menor osmolaridade e apresentarem maior palatabilidade em relação aos aminoácidos livres. Este dois fatores tornam estas proteínas ideais para uso na fabricação de suplementos alimentares para indivíduos com diversos tipos de doenças, por promover rápida absorção de peptídeos e reduzir o risco de diarreia (FRENHANI & BURINI, 1999).

Na década de 1990, quando se percebeu que o processo da digestão das proteínas tinha outras importantes atribuições fisiológicas, além de diminuir o tamanho das peptonas e facilitar a captação de aminoácidos e peptídeos pelos sítios de transporte no intestino, se entendeu que as proteínas alimentares podiam ser fontes exógenas de peptídeos bioativos (MEISEL, 2005). Desde então, as proteínas do leite bovino têm sido estudadas pela sua ação eminentemente anabólica e os peptídeos resultantes do processo digestivo estão começando a ser identificados pelas suas funções como sinalizadores celulares do metabolismo (MORIFUJI *et al*, 2009; MORATO *et al*, 2013).

Na busca de explicações para as vantagens fisiológicas observadas, quando as proteínas pré-hidrolisadas entram na dieta de um rato, em substituição da caseína, várias pesquisas têm apontado que existe um estímulo à síntese de glicogênio muscular, um efeito anti-estresse e um efeito citoprotetor (TASSI *et al*, 1998; PIMENTA *et al*, 2006; MORIFUJI *et al*, 2005 a,b; NERY-DIEZ *et al*, 2010; MOURA *et al*, 2013).

O estímulo à síntese de glicogênio muscular foi primeiro relatado em ratos que consumiam α -lactalbumina hidrolisada e eram submetidos ao exercício físico (TASSI *et al.*, 1998). Esse efeito foi mais tarde constatado de ocorrer também em ratos sedentários (FARIA *et al.*, 2012) que consumiam o hidrolisado das proteínas totais do soro.

3. Diabetes

Diabetes mellitus é uma doença metabólica caracterizada pela hiperglicemia resultante de defeitos na secreção e/ou na ação da insulina. (American Diabetes Association, 2012). Atualmente, o diabetes é classificado em tipo 1, tipo 2, gestacional e outros específicos, que são menos comuns (American Diabetes Association, 2012).

Atualmente o DM 2 é o tipo mais comum acometendo cerca de 90-95% dos diabéticos e está frequentemente associado a obesidade e acúmulo de gordura na região abdominal. Essa doença é caracterizada pela instalação da resistência a insulina que inicialmente é compensada pelo aumento na secreção desse hormônio pelas células β pancreáticas. A progressão dessa condição pode levar a redução da secreção da insulina decorrente da disfunção e morte das células β , e favorecer a hiperglicemia (Kahn *et al.*, 2006)

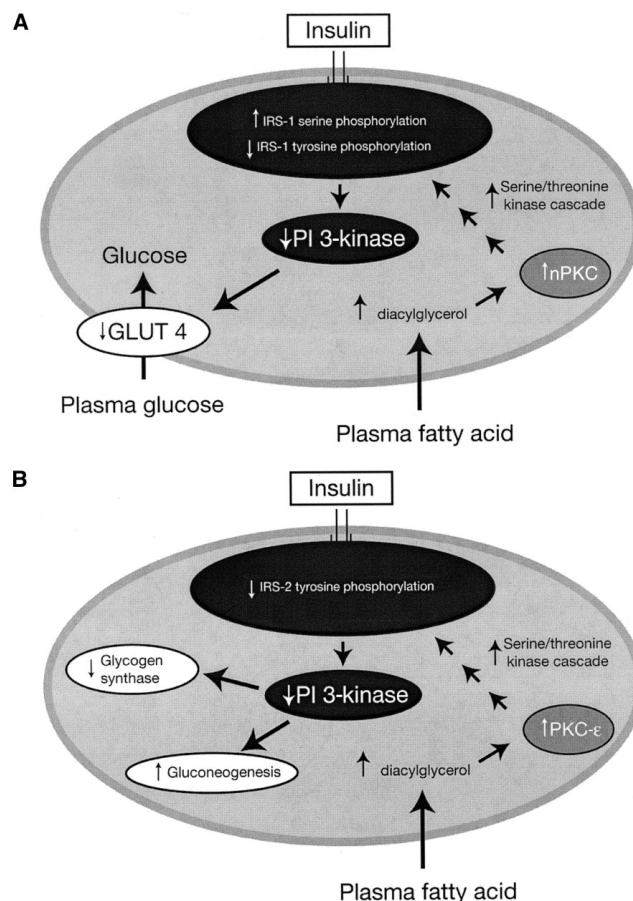


Figure 1- Mecanismo de resistência à insulina induzida por ácidos graxos no músculo (A) e fígado (B). GLUT 4 = transportador de glicose 4; IRS = substrato receptor de insulina; PI 3-kinase = fosfatidilinositol 3-quinase; nPKC = proteína C quinase. (PETERSEN & SHULMAN, 2006).

4. Benefícios atribuídos ao consumo das proteínas do soro do leite

4.1 Estímulo à secreção de insulina

As proteínas do soro do leite possuem capacidade insulínica, sendo eficientes em elevar a secreção de insulina após a sua ingestão quando comparada à proteína de soja (MORIFUJI *et al.*, 2010), ou mesmo quando comparada à caseína (POWER *et al.*, 2008). Dois fatores são apontados para esse efeito: a) a sua elevada digestibilidade proteica, seguida de consequentes altas concentrações de aminoácidos plasmáticos; b) perfil aminoacídico rico em aminoácidos essenciais, principalmente leucina, cuja capacidade insulínica já é bem conhecida (DA SILVA *et al.*, 2012).

É importante ressaltar que a redução na secreção de insulina afeta não apenas o metabolismo da glicose como também as várias vias relacionadas ao metabolismo de lipídios e aminoácidos, como captação de glicose, ativação da síntese de glicogênio, comprometimento da secreção de glicose produzida pelo fígado, aumento da gliconeogênese, comprometimento na supressão de lipólise e defeito na supressão de produção de lipoproteínas (TESSARI *et al.*, 2011).

Nilsson *et al.* (2004) ofereceu a 12 voluntários saudáveis, quatro tipos diferentes de dieta, sendo respectivamente leite em pó reconstituído, queijo, soro de leite, bacalhau e glúten em quantidades equivalentes de lactose e constatou que proteínas lácteas desempenham atividade insulínica e que a fração do soro de leite demonstrou ser um secretagogo de insulina mais eficiente que a caseína quando ingeridos em associação com carboidratos.

Outro estudo demonstrou que a proteína do soro de leite hidrolisada, ingerida sem o acompanhamento de carboidratos, causou um aumento significativo nos níveis plasmáticos de aminoácidos, dipeptídeos e insulina quando comparada com a proteína concentrada do soro de leite, proteína de soja e proteína hidrolisada de soja. Este rápido aumento pode ser explicado pela maior velocidade de esvaziamento gástrico desta proteína, que é maior que a caseína e a proteína de soja, e também por conter moléculas menores, o que facilita sua digestão. Estes achados podem indicar que o comprimento da cadeia peptídica de hidrolisados de proteínas pode afetar as respostas, aumentando os níveis plasmáticos de aminoácidos (MORIFUJI *et al.*, 2010).

Em animais experimentais, estudos indicam que o consumo de proteínas do soro do leite possuem um efeito insulínico, por si só, que estimulam a secreção de insulina pela atuação direta nas células β (JAKUBOWICZ & FROY, 2012). Esta propriedade parece estar relacionada a altos teores dos aminoácidos isoleucina valina, lisina, treonina e, principalmente leucina e peptídeos contendo leucina presentes nestas proteínas (MORATO *et al.*, 2013).

4.2 Prolongamento da resposta insulínica e controle da glicemia

O peptídeo semelhante ao glucagon-1 (GLP-1) é um hormônio multifuncional liberado após as refeições, que estimula a liberação de insulina. Indivíduos com diabetes apresentam baixos níveis de GLP-1. Uma das enzimas que hidrolisa as incretinas, como o GLP-1, é a enzima dipeptidyl peptidase-IV (DPP-IV). A inibição dessa enzima em ratos tem mostrado suprimir a elevação da glicose sanguínea (TAKASAKI *et al.*, 2004). Estudos de dois diferentes grupos de pesquisadores (GUNNARSSON *et al.*, 2006; DRUCKER, 2006) mostram a capacidade das PSL em inibir a DPP-IV e elevar os níveis de GLP-1, resultando em aumento e prolongamento da resposta à insulina em camundongos e consequente controle glicêmico.

Em outro trabalho com camundongos obesos, a suplementação com proteínas do soro do leite hidrolisadas aumentou a taxa de mobilização (*clearance*) da glicose, reduziu a hiperinsulinemia e restaurou a capacidade das ilhotas pancreáticas de secretar insulina em resposta à aplicação de uma dosagem de glicose de 2 g/kg de peso corporal (GAUDEL *et al.*, 2013).

4.3 Promoção da saciedade e controle ponderal

Hall e colaboradores (2003) mostraram em humanos que a saciedade após o consumo de PSL é maior que a proporcionada pela caseína, acompanhada por maiores níveis plasmáticos de colecistoquinina em cerca de 60%. A colecistoquinina é liberada pelo duodeno e pode contribuir para o fim da refeição com a sensação da saciedade (KISSILEFF *et al.*, 1981)

Outra hipótese, levantada por Zhou *et al.* (2011) é a da regulação hipotalâmica do apetite, via inibição da expressão do Peptídeo Relacionado ao Agout (AGRP), peptídeo orexígeno expresso no núcleo arqueado do hipotálamo. A proteína do soro do leite contém uma das maiores concentrações de aminoácidos de cadeia ramificada (BCAAs) e de L-leucina. A leucina atravessa a barreira hematoencefálica mais rapidamente que os

demais aminoácidos, provocando a inibição da síntese do peptídeo AGRP e, consequentemente, levando à saciedade.

Após ingestão de bebida contendo 50g de PSL, indivíduos apresentaram supressão prolongada de grelina e elevação de GLP-1 e CCK, quando comparados com indivíduos que ingeriram bebidas contendo 50g de frutose ou 50g de glicose (BOWEN *et al.*, 2007).

Utilizando um sistema de gravação automática informatizada, o padrão alimentar de camundongos foi monitorado por Yu *et al.* (2009) durante sete dias. Quanto aos animais que ingeriram a dieta com proteína do soro do leite, o intervalo entre as refeições foi maior; o número de refeições por dia foi menor comparado àqueles alimentados com dietas cujas fontes proteicas foram glúten e soja.

4.4 Melhora do perfil lipídico

De acordo com Jimenez (2003), fatores como dislipidemias com predominância da hipertriacilglicerolemia, descontrole glicêmico e a hipertensão arterial parecem ser os pré-requisitos para o surgimento da disfunção endotelial e, consequentemente, do surgimento de doenças cardiovasculares.

Uma única dose de 45 g de PSL reduziu significativamente os triacilgliceróis plasmáticos pós-prandiais, medidos pela área sob a curva, quando comparado com 45 g de caseína ou glicose em mulheres obesas pós-menopausa (PAL *et al.*, 2010). O mesmo grupo de pesquisadores, em experimento crônico, observou que o consumo de PSL durante 12 semanas por indivíduos obesos foi capaz de reduzir os níveis de triacilgliceróis de jejum quando comparado com o grupo controle e também os níveis de colesterol total e LDL-c quando comparado com o grupo que recebeu caseína (PAL *et al.*, 2010).

Kawase *et al.* (2000) também observou melhora no perfil lipídico de voluntários que consumiram suplemento de PSL adicionado ao leite

fermentado por 8 semanas. Para os possíveis mecanismos, Zhang & Beynen (1993) sugerem que as PSL poderiam influenciar a biogênese de colesterol no fígado, enquanto Nagaoka e colaboradores (1992) apontam um efeito das PSL em inibir a absorção de colesterol no intestino e Chen & Reimer (2009) encontraram um efeito das PSL na redução na expressão de genes envolvidos na síntese e absorção ácidos graxos e colesterol *in vitro*.

4.5 Efeito anti-estresse

As proteínas do soro do leite também têm sido objeto de estudo pelo seu efeito protetor dos tecidos do organismo. Em ensaios com ratos Wistar, Castro et al. (2010) e Rosanelli *et al.* (2004) mostraram que úlceras intestinais induzidas eram amenizadas pelo consumo de proteínas do soro de queijo.

O trato gastrointestinal é o principal órgão de utilização da glutamina. Os enterócitos, células do intestino, são células de alta taxa de divisão celular e necessitam de glutamina e glicose como fonte de energia (POMPÉIA, 2000). Em situações de estresse, o organismo apresenta uma alta concentração de glutamina na corrente sanguínea, resultante do aumento da sua captação no intestino (SOUBA *et al.*, 1990), portanto, na ausência do estresse, a atividade da glutaminase é diminuída.

Estudos realizados nos laboratórios da Unicamp mostraram que a ingestão das proteínas do soro do leite resulta em efeitos benéficos, tanto em ratos exercitados, quanto sedentários. Frente ao exercício físico exaustivo, Nery-Diez *et al.* (2010) propuseram que o consumo das proteínas do soro do leite hidrolisadas reduzem o estresse produzido pelo exercício, evidenciado pela redução da atividade da glutaminase intestinal.

As heat shock proteins (HSPs), proteínas sintetizadas pelo organismo, inicialmente reconhecidas em situação de choque térmico em moscas *Drosophila melanogaster* (RITOSSA, 1962), são hoje conhecidas como proteínas do estresse. Elas auxiliam na manutenção da homeostase orgânica, em situações adversas como: aumento de temperatura, estresse oxidativo,

estresse osmótico, hipóxia, isquemia e reperfusão, privação de glicose, processo inflamatório entre outros (KLINE *et al.*, 1997).

As HSPs podem também ser consideradas como um sistema antioxidante complementar, auxiliando na defesa do organismo contra as espécies reativas de oxigênio (EROs) (ZOPPI *et al.*, 2004). Portanto elas vêm sendo consideradas como um mecanismo natural de defesa endógena primária que é capaz de proteger e reparar danos causados, conferindo à célula uma tolerância contra uma variedade de agentes agressores.

Resultados de nosso grupo mostram claramente que as PSLH podem elevar os níveis de HSP-70 (MOURA *et al.*, 2012), o que pode conferir uma maior tolerância do organismo quando alimentado com essas proteínas ao estresse oxidativo ou mesmo inflamatório.

Segundo a literatura atual sobre os efeitos do consumo das proteínas do soro do leite, tanto intactas quanto hidrolisadas, nota-se certas propriedades especiais, as quais podem ser divididas em dois tipos: um, relacionado ao fato de estas proteínas terem sido projetadas pela natureza para o crescimento dos mamíferos e, outro, relacionado aos hidrolisados. Este último ilustra um dos mecanismos adotados pela natureza para magnificar a funcionalidade de biomoléculas, atribuindo-lhes múltiplas funções bioquímicas ou metabólicas. No caso das proteínas do soro do leite, as funções adicionais dos hidrolisados são percebidas nos estados de estresse, na modificação da capacidade de captação da glicose e na sinalização celular, que é uma forma de regulação do metabolismo em geral.

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CHAPTER 2

**IMPACT OF PRE-HYDROLYZED MILK WHEY PROTEIN INTAKE
ON INDUCED DIABETES MELLITUS IN RATS**

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ABSTRACT

The occurrence of diabetes is associated with obesity and abdominal fat accumulation, and the intake of milk whey proteins has been suggested to improve glucose metabolism and body composition. In order to evaluate the ability of the whey proteins on minimizing or preventing the effects caused by a high-fat (HF) diet plus treatment with streptozotocin (STZ). Eight-week old Wistar rats were fed an HF-diet containing different protein sources, namely: casein (CAS), milk whey concentrate (WPC) and pre-hydrolyzed WPC (WPH). Another control group consumed the standard AIN 93-G diet. After 21 days, the experimental groups received a dose of 40 mg/kg of streptozotocin (STZ) and were sacrificed 10 days later. The HFWPH group showed lower weight gain and lower AUC in the glucose tolerance test among experimental groups. However, the parameters of aspartate transaminase (AST), alanine transaminase (ALT), triacylglycerols (TAG) and hepatic fat were all higher in the groups receiving the HFWPC and HFWPH diets. It is concluded that consumption of whey protein, whether intact or pre-hydrolyzed, in spite of mitigating the excessive weight gain and insulin resistance, was not able to prevent or reverse the damage caused by HF diet-STZ model. On the other hand, the classic parameter of liver function, ALT and serum triacylglycerols suggested the existence of a possible antagonism between the use of hepatotoxic streptozotocin and whey proteins.

Keywords: diabetes, whey, hydrolyzed protein, obesity, insulin resistance, hyperglycemia, dysglycemia

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in the secretion and/or action of insulin. The worldwide prevalence of this condition is increasing and, according to the International Diabetes Federation (2012), more than 371 million people suffered from diabetes worldwide. The countries that lead the ranking are China, India, USA and Brazil. In addition, about 4.8 million people died from complications of the disease only in 2012.

Type-2 diabetes mellitus (DM2) is currently the most common type of the disease, affecting approximately 90-95 % of diabetic patients and is often associated with obesity and fat accumulation in the abdominal region (Kahn *et al.*, 2006).

The DM2 caused by hyperglycemia is a result of the reduction of glucose uptake by insulin-dependent tissues such as muscle and adipose tissue, increased gluconeogenesis in the liver, and reduction of insulin secretion caused by the lower mass of β cells in pancreatic islets (Petersen & Shulman, 2006). Studies on the role of insulin resistance in body fat show that more important than the absolute amount of fat in the body, is the intracellular lipid content in liver and skeletal muscle. Obesity caused by caloric intake above the energy requirement leads to fat accumulation not only in adipocytes, but also in liver and muscle tissues, which results in insulin resistance in these organs (Petersen & Shulman, 2006).

Recent research in food and nutrition has sought to deepen the scientific understanding of the essence of food in order to reassess the role of human diet in the prevention and treatment of chronic diseases, including type 2 diabetes. Accordingly, many studies indicate that there is a wide range of chemicals and nutrients, such as phenolics, carotenoids and various precursor proteins of bioactive peptides, which have shown some power in the prevention or treatment of diseases such as diabetes mellitus (Jackix *et al.*, 2013; Trigueros *et al.*, 2013; Mousavi *et al.*, 2013; Sharma, 2006).

The whey is obtained from the acid or enzymatic coagulation of the caseins during cheese manufacturing (Gentleman, 2007). The whey protein content corresponds to about 20% of total milk protein and is composed mainly by the soluble proteins β -lactoglobulin, α -lactalbumin, bovine serum albumin, lactoferrin, immunoglobulins and the disaccharide lactose, and minerals (Marshall, 2004). These proteins contain high concentrations of branched chain amino acids (BCAAs), namely leucine, isoleucine and valine (Bowen *et al.* 2006), and a reasonable content of sulfur amino acids (methionine and cysteine).

The whey proteins are easily digested and are more rapidly absorbed, thus promoting greater aminoacidemia after intake, compared to casein (Boirie *et al.*, 1997). They are not only good sources of amino acids, but also a source of bioactive peptides generated during the digestion process. These peptides bind to receptors in the intestinal lumen before absorption, or to receptors of target organs after absorption into the bloodstream (Luhovyy *et al.*, 2007). Among other properties, the research group at Unicamp has shown that ingestion of a whey protein hydrolyzate (WPH) can result in increased translocation of inactive glucose transporter-4 (GLUT-4) from the cytoplasm to the cell membrane of skeletal muscle, thus increasing glucose uptake by muscle tissue (Morato *et al.*, 2013).

In another work by the Unicamp group, it was also shown that consumption of WPH can increase the expression of endogenous proteins named chaperonins or heat shock proteins (HSPs), which are responsible for the protection of tissues against various types of stress, including stress caused by excessive heat, exercise and chemical stress (Moura *et al.*, 2013).

As a result of the discovery of the above-mentioned new properties, the present study sought to evaluate and compare the influence of the consumption of whey protein in its two forms, intact (WPC) and hydrolyzed (WPH), in preventing or ameliorating the development of diabetes induced by a high-fat diet, in addition of the administration of the antibiotic streptozotocin (40 mg / kg) in rats.

2. MATERIALS AND METHODS

2.1 *Experimental design*

Forty male Wistar specific pathogen free (SPF) 21 days old rats and weighing approximately 50g, were ordered from the Multidisciplinary Center for Biological Investigation (CEMIB, Universidade Estadual de Campinas-UNICAMP). The rats were housed in individual cages in the Biological Testing Laboratory (LEB) in the Department of Food and Nutrition (DEPAN), in which environmental conditions were controlled to maintain the temperature at $22 \pm 2^{\circ}\text{C}$, relative humidity 50-60% and light/dark the normal 12-hour cycle. Filtered water and diet were offered *ad libitum*. This experiment was approved by the Ethics Committee on Animal Experimentation (CCEA-IB - UNICAMP) under No. 2913-1/2012 protocol (ANNEX 1). The animals were fed a commercial diet (Labina, Curitiba, PR, Brazil) for 5 weeks (8 weeks of age) and randomized in order to start the experimental feeding *ad libitum* for 31 days. At the end of the experimental period, the animals were sacrificed after 8 hours of controlled fasting. Blood, hepatic and epididymal fat were extracted and carcasses and livers frozen at -20°C for further analysis. The animals were divided into diet-groups according to the lipid content and protein source, as shown below in Figures 1 & 2.

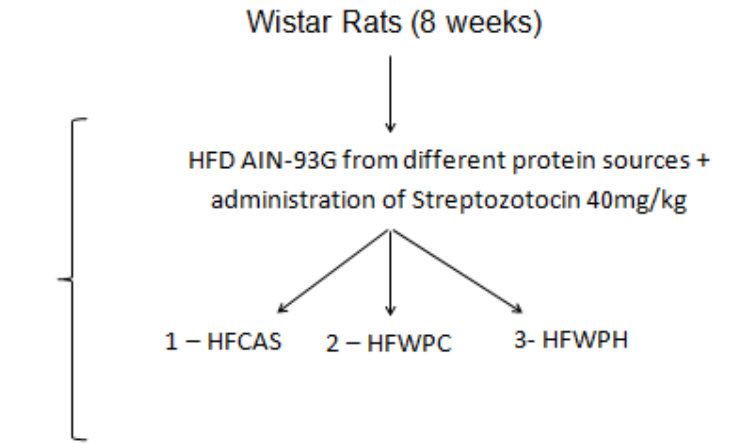


Figure 1- Experimental design. 2 – AIN-93G with casein and high fat; 3 – AIN-93G with whey protein concentrate and high fat (HFWPC). 4 – AIN-93G with hydrolyzed whey protein and high fat. The diets were eaten during 31 days.

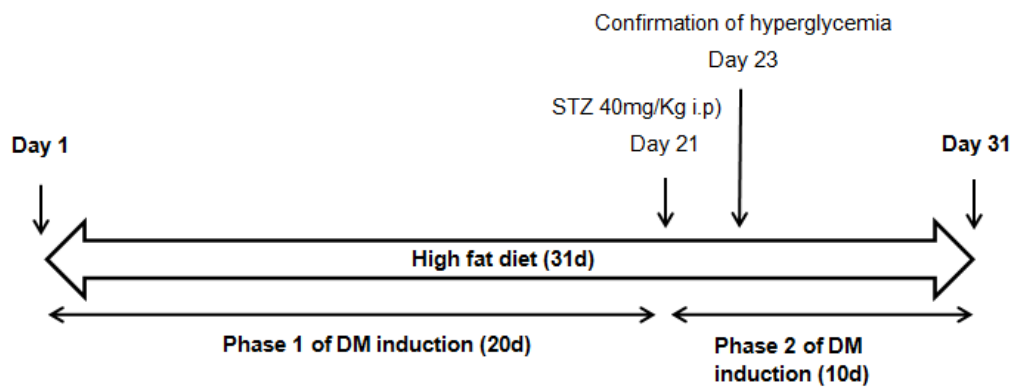


Figure 2- Experimental time-course. Phase 1: induction with a high-fat diet. Phase 2: reinforcement of induction with an injection of streptozotocin.

2.2 Diets

Control diets were formulated according to the recommendations of the AIN 93-G, and the experimental diets were adjusted in relation to the control

following the same recommendations (Reeves et al., 1993) and using the same percentages of protein and lipid content of Cintra et al. (2008). Whey protein from milk, in both forms, concentrate and hydrolyzate (Hilmar 8000™ and 8350™, respectively (Hilmar Ingredients; Hilmar, CA, USA), and casein as standard were used as protein sources. For calculating the desired percentage of protein of 20% in the diets, protein contents were determined by the method of Kjeldahl described by the AOAC (2005) using the conversion factor of 6.38 for all proteins. After preparation of the diets, new analyses were performed to confirm the composition of protein, fat and ash, again according to the AOAC (2005), except for lipid, when the method of Bligh and Dyer (1959) was used.

Table 1- Diet composition

Ingredients	Standard	High fat		
	(Reeves et al., 1993)	(Cintra et al., 2008)		
	(AIN-93G) Casein (90% protein)	Casein (90% protein)	Concentrated Whey (81% protein)	Hydrolysate Whey (79% protein)
Protein source	222,22	222,22	246,91	253,16
Corn starch	383,45	205,39	189,80	185,85
Dextrinized cornstarch	127,34	68,21	63,03	61,72
Sucrose	96,47	51,67	47,75	46,76
L-cystine	3,00	3,00	3,00	3,00
Fiber (microcelulose)	50,00	50,00	50,00	50,00
Soybean oil	70,00	40,00	40,00	40,00
Lard (pork)	-	312	312	312
Mineral mix (AIN93G – mix)	35,00	35,00	35,00	35,00
Vitamin mix (AIN93G – mix)	10,00	10,00	10,00	10,00
Choline bitartrate	2,50	2,50	2,50	2,50

2.3 Induction of diabetes with streptozotocin (STZ)

Although there are several methods to induce diabetes (Islam & Wilson, 2012), the method involving the combination of a diet high in fat and the use of the antibiotic streptozotocin (STZ) was selected because it is still widely used and recommended for being practical and reproduce the natural pathogenesis with production of various characteristics related to the development of T2DM, that is body fat accumulation and subsequent development of insulin resistance.

After three weeks of consuming the experimental high fat diets, all animals were subjected to 8 hours of fasting. Those consuming the HF diet received an intraperitoneal injection of 40 mg/kg streptozotocin (Sigma, St. Louis, MO, USA) diluted in sodium citrate buffer at pH 4.5. The control group received an injection of only the buffer. At 72 hours after the injection, the animals entered a 6-h fasting to confirm hyperglycemia, determined with the use of a glucometer (Accu Check Active, Roche, Germany). Besides hyperglycemia, animals that responded to the induction showed polyuria, polydipsia, and those that did not show the characteristic symptoms were excluded from the study.

2.4 Analyses

2.4.1 Body weight control – The animals were weighed once a week, on the same day of the week and at the same time, around 9 am.

2.4.2 Food intake control – Diets were changed three times per week to prevent lipid oxidation. Leftovers in feeders and trays were separated from feces and weighed. Daily diet intakes were estimated by subtracting leftovers from the amounts served.

2.4.3 Determination of food and energy efficiency – In order to determine the biological value of diets, the energy efficiency index, which estimates the efficiency of energy intake in causing weight gain in animals (Yu *et al.*, 2004) was used. At first, the dietary intake (g/day) was multiplied by the energy content of the diet (kcal/g) to obtain the daily caloric intake per animal, and subsequently, the energy efficiency was calculated as: (weight gain / kcal) x

100. This test was of interest in our study because of the impact associated with high fat intake and the possible effect on triad-accumulation, intake-energy expenditure in animals.

2.4.4 Glucose tolerance test (ipGTT) – One week before the application of STZ, the animals underwent 6h of fasting, were weighed and had fasting glucose level (zero time) checked via tail with the help of a glucometer (Accu Check Active, Roche, Germany). Then the animals received an intraperitoneal injection of glucose at concentration of 2 g/kg body weight. Blood glucose was checked again at 30, 60, 90 and 120 minutes after application of glucose.

2.4.5 Analyses by commercial kits – In order to obtain a comprehensive picture of the potential effects of replacing the protein source of the diet on the metabolism of the animal, monitoring the possible alterations of classical parameters were considered important. Therefore, the following serum parameters: albumin, AST, ALT, total cholesterol, HDL cholesterol, glucose, total protein and triacylglycerols were determined by commercial kits (Laborclin Products Laboratory Ltd., Pinewoods, PR, Brazil).

2.4.6 Insulin determination by radioimmunoassay (IRA) – At sacrifice, blood was withdrawn and centrifuged at 3000 rpm, for 10 min at 4°C. About 100µL were utilized for dosing insulin by radioimmunoassay (SCOTT *et al.*, 1981).

2.4.7 Proximate carcass composition and relative weights of organs – After removal, gastrointestinal tracts were washed with distilled water, and returned to each carcass, which in turn were weighed and frozen at -20 °C for two weeks. After this period, while still frozen, slicing was done and the resulting material was lyophilized. The lyophilized carcasses were ground to obtain a homogeneous powder, for further analysis. All analyses were performed in triplicate.

The following determinations were performed: total protein by Kjeldahl, body fat percentage and percentage of hepatic fat by the method of Bligh &

Dyer (1959) and total ash, all in accordance with the procedures described by the AOAC. For protein determination, the conversion factor of 6.25 was used.

The relative organ weights were calculated by multiplying the organ weight by 100 and dividing this value by the weight of the carcass without the blood ((organ weight x 100)/weight of carcass).

2.4.8 Determination of liver fat – After sacrifice, livers were weighed and stored at -20 °C. For determination of the lipid content, the livers were thawed to room temperature, macerated until complete homogenization and proceeded to the determination of lipid by the method of Bligh & Dyer (1959).

2.5 Statistical treatment

Data were analyzed using SPSS 18.0 software for Windows. Data in tables are presented as mean $\pm$ SP. Comparisons were performed using one-way ANOVA with post-test of Duncan. Values were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Induction of hyperglycemia with the hyperlipidic diet and streptozotocin (STZ)

As expected, animals fed the high-fat diet and treated with streptozotocin (40 mg/kg body weight) showed a significant body weight reduction in phase 2, and increased levels of blood glucose, water consumption (polydipsia) and volume urine (polyuria), when compared with the normolipidic diet group which received only the vehicle citrate (Srinivasan et al., 2005). Of the total, 6 animals died, 4 animals being from the HFWPC group.

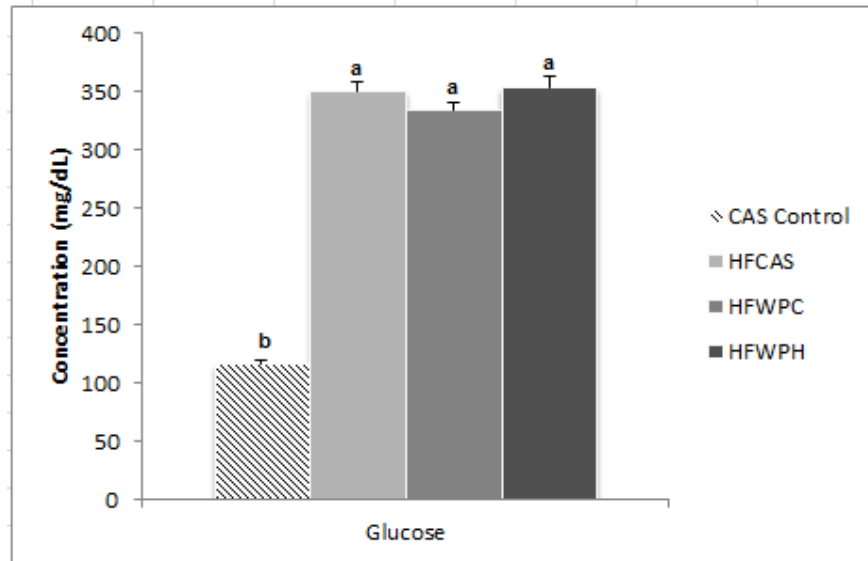


Figure 3 -Glucose 72 hours after STZ administration. The values represent the means $\pm$ SE. Different superscript letters indicate statistical differences ($p < 0.05$) $n=6$.

3.2 Body mass evolution

In phase 1 of the experiment, weight gain was greater in groups HFWPC and HFCAS in relation to groups CAS CONTROL and HFWPH, which showed apparently normal weight gain ($p < 0,05$).

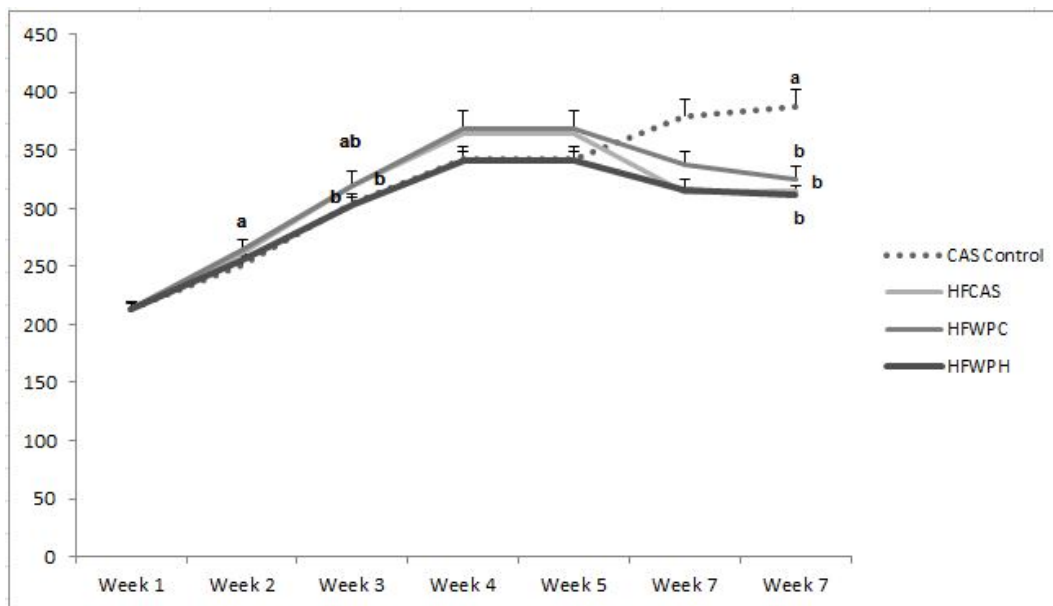


Figure 4 – Body weight gains of phase 1 and 2 of DM induction. Growth curves for groups HFWPC and HFCAS coincided on the upper line, while CAS Control and HFWPH coincided on the lower line. After DM induction, occurs loss weight in 3 groups injected STZ. Values represent the means $\pm$ SE $n=6$.

In phase 2, the pattern of response to diet was reversed, while the CAS Control group showed greater weight gain than the other groups ($p < 0:01$).

The ability to control body weight promoted by the whey protein in rodents fed a high fat diet has been proposed by some researchers (Shertzer *et al.*, 2011; Shi *et al.*, 2012; McAllan *et al.*, 2013). However, this effect was only observed for the hydrolyzed protein (PSLH), and not for the non-hydrolyzed (PSL). The process of hydrolysis of the whey proteins leads to the generation of peptides of various sizes. Studies indicate that once formed, some of these peptides express bioactivity, and one of the functions of bioactive peptides is to assist in the modulation of energy metabolism, thereby promoting higher glucose uptake, and subsequent higher glycogen synthesis (Morifuji *et al.*,

2009; Morato *et al.*, 2013). Thus it would be reasonable to think that consumption of whey hydrolyzates could assist in the prevention of obesity and insulin resistance.

3.3 Diet intake

Dietary intake in the CAS Control group was greater than in the other groups in both phases of the experiment ($p < 0.01$).

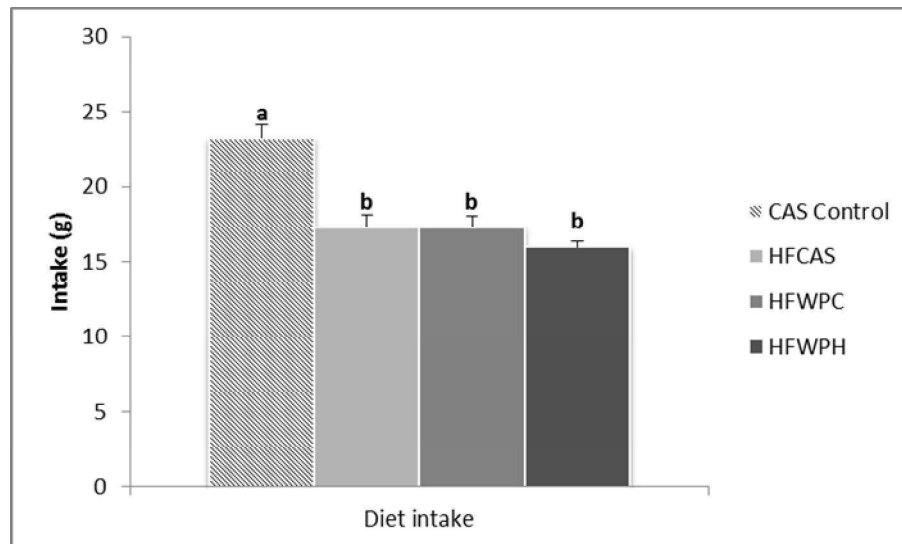


Figure 5 - Mean daily dietary intake during phase 1 of DM induction. Values represent means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$) $n=6$.

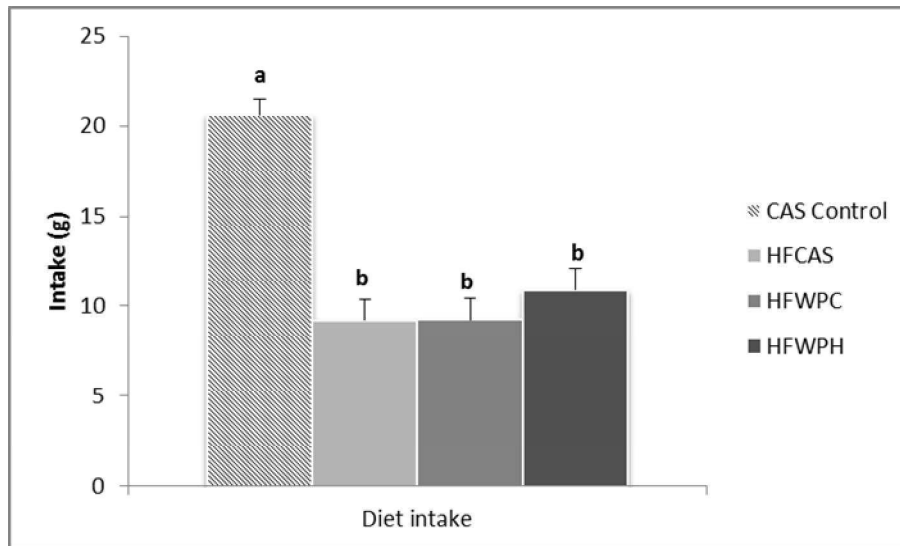


Figure 6- Mean daily dietary intake during phase 2 of DM induction. The values represent the means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$) $n=6$.

3.4 Energy efficiency

Energy efficiency in phase 1 was higher in groups HFWPC and HFCAS, intermediate in HFWPH group and lowest in the standard Casein Control group ($p < 0.01$). In phase 2, the CAS Control group had higher efficiency values than the others ($p < 0.01$).

After calculating the energy efficiency of diets, it was observed that, even with no significant difference between the mean intake of a high fat diet, there was a trend to lower efficiency of HFWPH diet, suggesting that whey protein hydrolyzate promotes greater thermogenesis compared to other protein sources (Froy & Jakubowicz, 2012). Regarding the data on weight gain and energy efficiency in phase 2, it was also expected that this would happen because weight loss is a result of the implementation of streptozotocin (Srinivasan et al., 2005).

The superiority of whey protein over other protein sources to promote higher concentrations of CCK (cholecystokinin) has been demonstrated by some authors (Kissileff et al, 1981; Hall *et al.*, 2003), which would cause a

decrease in food intake, but our study showed no difference in consumption between the different protein sources studied.

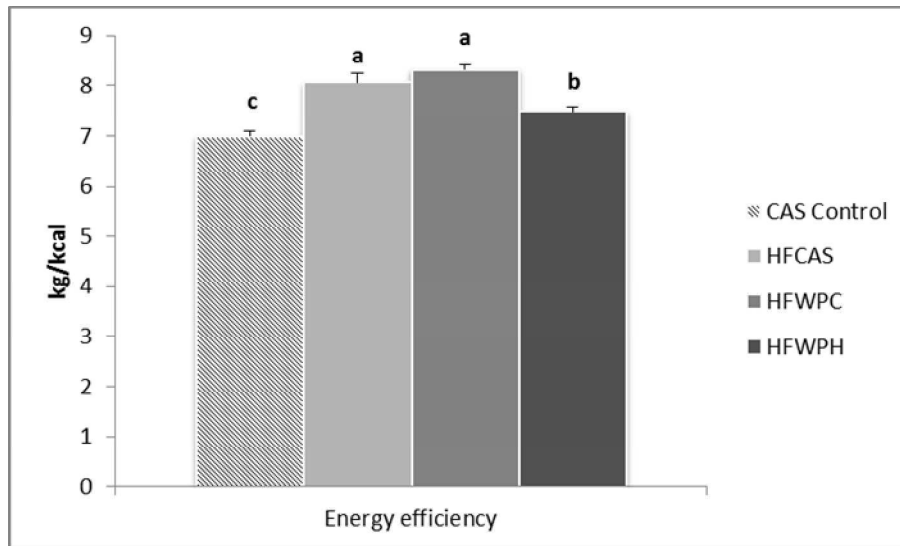


Figure 7 - Food efficiency during phase 1 of DM induction. The values represent the means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$) $n = 6$.

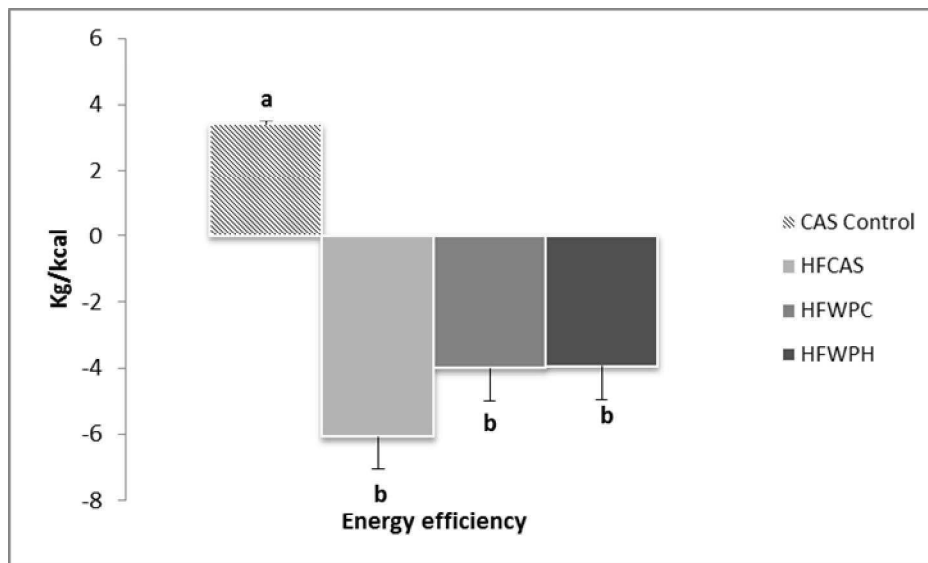


Figure 8 - Food efficiency during phase 1 of DM induction. The values represent the means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$) $n = 6$.

3.5 Glucose tolerance Test (GTT)

In calculating the area under the curve values HFCAS, HFWPC and HFWPH were higher, but in groups HFCAS, HFWPC values were slightly higher and statistically different from HFCAS, HFWPC.

As to the values of glucose tolerance test of the animals in group HFWPH being similar to those of the CAS Control is an outcome that may be attributed to the ability of whey protein to inhibit the enzyme dipeptidyl peptidase IV (DPP-IV) leading to increase in the concentrations of glucagon-like peptide (GLP-1), a hormone released after eating that promotes increased secretion of insulin, thus improving glycemic response (Gunnarsson *et al.*, 2006; Drucker, 2006). Recent studies have shown that increasing GLP-1 (glucagon-like peptide-1) is connected not only to glycemic control, but also to weight loss in obese, diabetic or nondiabetic subjects (Vilsbøll *et al.*, 2012).

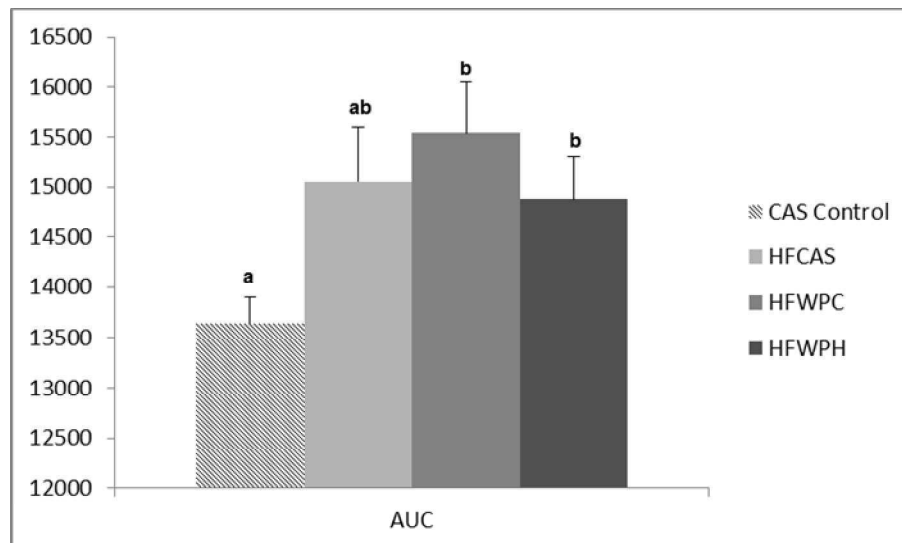


Figure 9 - Area under curve (AUC) of glucose tolerance test (GTT) – at the end of phase 1 of DM induction. The values represent the means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$). $n = 10$.

3.6 Adjusted weights of organs

The Figure 10 shows that the adjusted epididymal fat and carcass weights did not differ between groups, while the relative liver weights were higher in groups HFCAS, HFWPC and HFWPH, relative to CAS Control ($p < 0.01$).

Gasparetto *et al.* (2013) observed mass reduction of the peritoneal and epididymal adipose tissue but not in the total fat, in normal healthy Wistar rats that consumed diet containing whey protein concentrate for a period of 2 weeks. In our study, reduction of the mass of epididymal fat was not observed as a result of modifying the protein source to counteract the high dietary fat intake. The carcass weights did not differ either, because although animals fed a high fat diet have gained more weight in phase 1, the application of STZ led to weight loss of the treated groups, causing an approximation of the groups with respect to the final weights. The increase in adjusted liver mass was also observed by Hamad *et al.* (2012) and was attributed to the drug hepatotoxicity (Hamadi *et al.*, 2012; Juśkiewicz *et al.*, 2008.).

3.7 Chemical composition of the carcasses

The percentages of protein, lipid and ash (Figure 11) showed no significant differences between groups ($p > 0.05$). The levels of liver fat had to be superior in all groups treated with HF ($p < 0.01$) diet. However, in groups HFWPC and HFWPH, hepatic fat values were slightly higher and statistically different from group HFCAS.

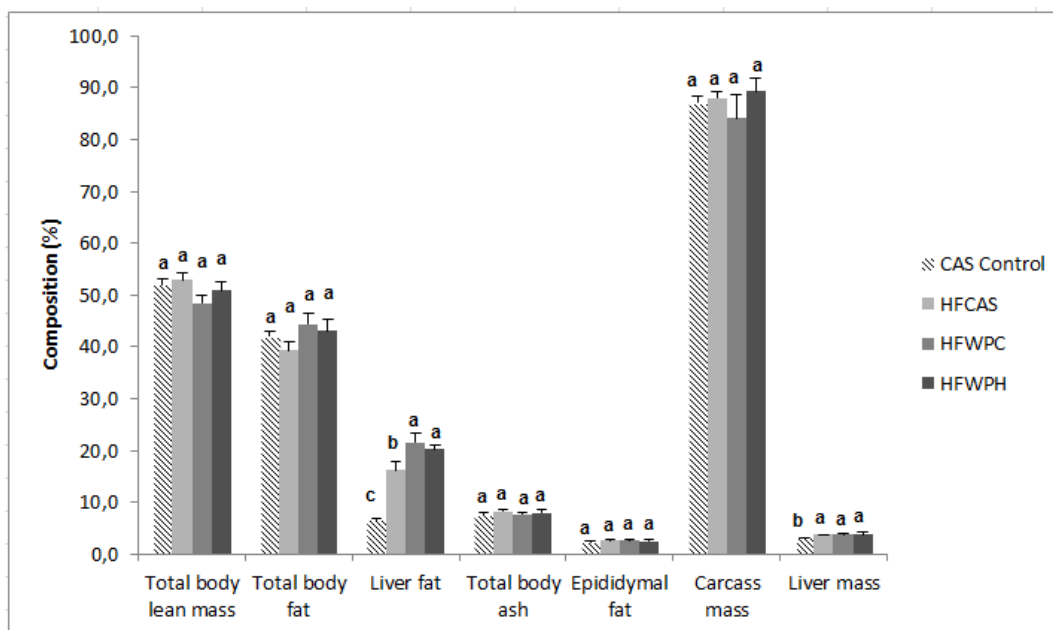


Figure 10 - Body composition at the end of phase 2 of DM induction. The values represent the means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$) $n=6$.

3.8 Biochemical parameters

The values of fasting glucose at the end of the experiment were elevated in all groups with induced DM, as was to be expected. However, the HFWPC group stood out with the highest elevation. This group did not differ from groups HFWPH and CAS CONTROL (Figure 12). No differences in total cholesterol levels were found, but concentrations of HDL were highest in the CAS Control group ($p < 0.05$). As far as the triacylglycerols, all groups showed substantial DM-induced elevation of TAGs, although this increase was significant only for groups HFWPC and HFWPH ($p < 0.05$).

Inspection of the profile of biochemical parameters of the animals confirmed the hepatotoxicity caused by STZ, as can be gathered from high ALT and TAG values, as well as the elevated hepatic fat contents. It was interesting to note, however, that the values of ALT and TAGs were particularly higher in

the groups consuming the whey protein in its two forms (intact and hydrolyzed). As of this time, we have no satisfactory explanation for this unprecedented result, but one possibility is that the combination of a high-fat diet treatment, followed by the application of a hepatotoxic drug, directs the cell-signaling properties of peptides from whey towards an unsuspected harmful effect. It is also pertinent to emphasize that the negative effect was present in all hyperlipidemic diet groups, although most notably negative in ALT and the triacylglycerols, and particularly for the serum proteins, while changes in AST were not significant.

Regarding the possible protective effect of whey the protein hydrolyzate (WPH) against tissue damage caused by diet and drugs in the diabetic animal, it can be seen that although all rats showed increased blood glucose levels of approximately 400%, the animals fed the WPC and WPH showed to have surpassed the levels found in animals fed casein (Figure 12). Nevertheless, the difference was significant only between the means of casein and WPC, suggesting that the WPH offered some protective effect. This tendency held some consistency with the observed resistance in the GTT test, when animals were treated with the HF diet only (Figure 11).

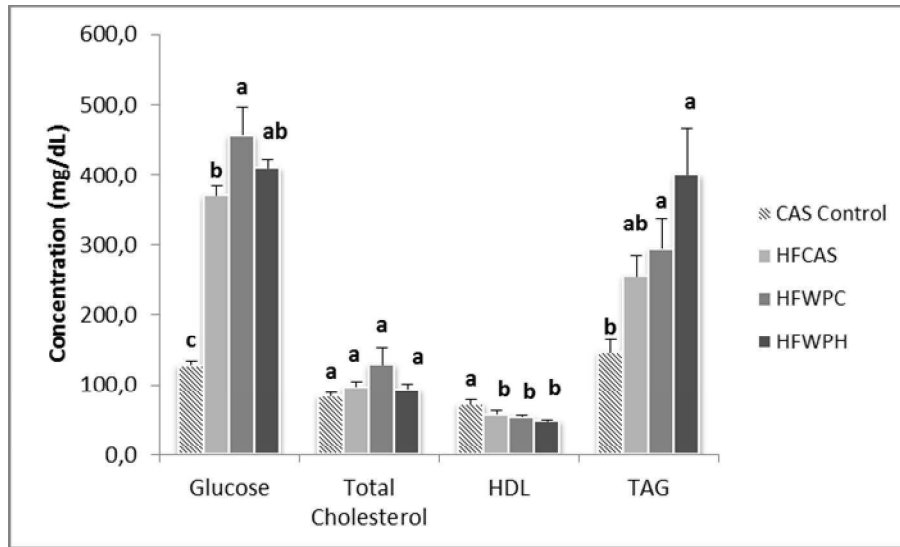


Figure 11 - Serum glucose, total cholesterol, HDL and triacylglycerols at the end of phase 2 of DM induction. Values are means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$) $n=6$.

No difference was observed in serum proteins, except albumin HFWPH group, relative to CAS Control. The weight loss in this group in phase 2 was similar to the other groups treated with STZ. However, since weight gain in phase 1 was the lowest among the experimental groups, protein catabolism generated by the drug may have contributed to this result, but no difference was observed in the determination of total serum.

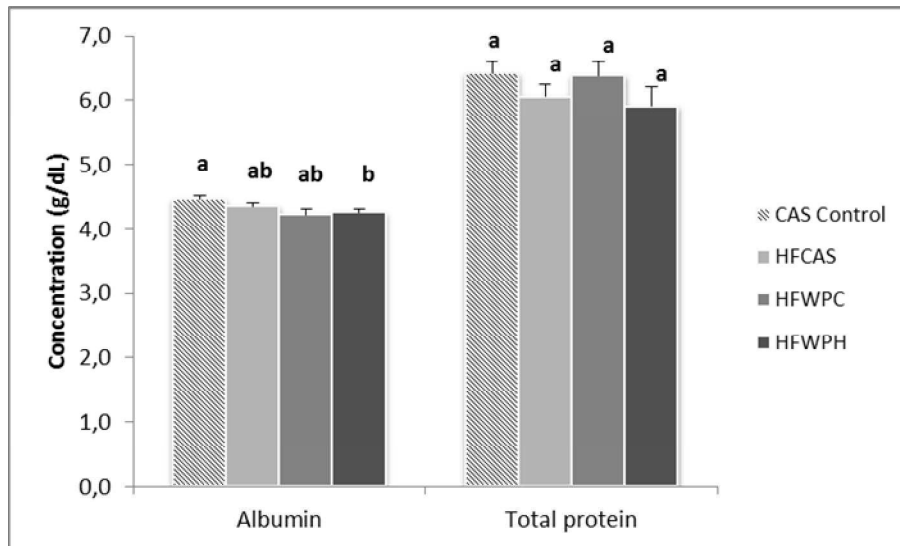


Figure 12 – Serum albumin and total protein at the end of phase 2 of DM induction. Values represent means $\pm$ SE. Different letters on bars indicate statistical differences ($p < 0.05$) $n=6$.

With the induction of DM, there was an increase in ALT (Figure 14) in animals with induced DM, being significant in groups HFWPC and HFWPH, relative to CAS Control. The AST did not differ significantly between groups ($p > 0.05$). Insulin levels (Figure 15) were higher only in the group CAS Control in relation to the others ($p < 0.002$).

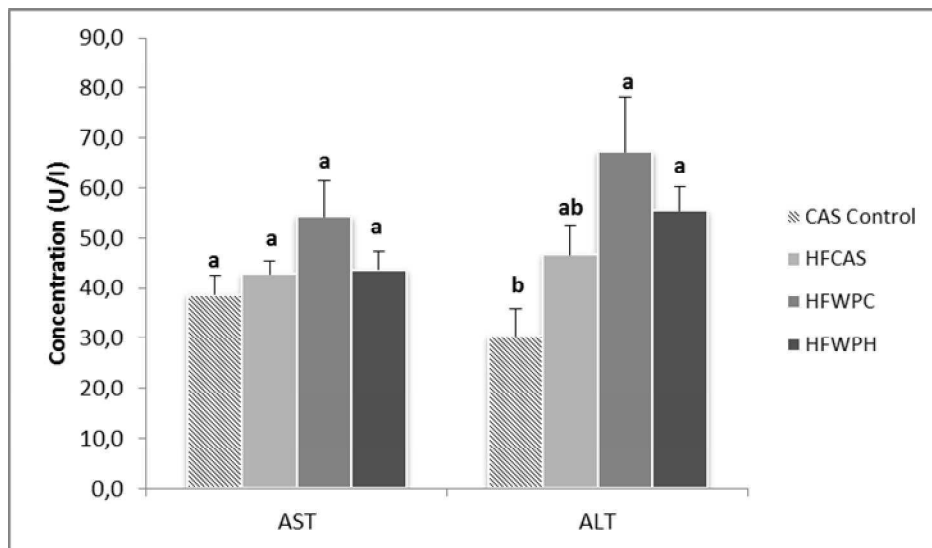


Figure 13 - Serum AST and ALT at the end of phase 2 of DM induction. The values represent the means $\pm$ SE. Different letters on bars indicate statistical differences ($p<0.05$) $n=6$.

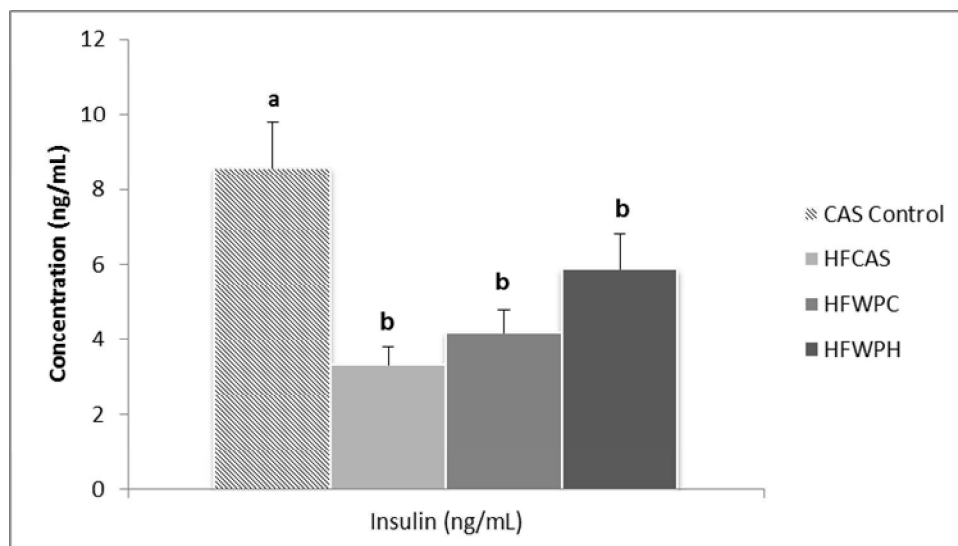


Figure 14 - Insulin at the end of phase 2 of DM induction. The values represent the means $\pm$ SE. Different letters on bars indicate statistical differences ($p<0.05$) $n=5$.

Table 2 - Biochemical parameters in mean $\pm$ SE of the mean n=6

Experimental groups				
Parameters	CASEIN CONTROL	HF CAS	HF WPC	HF WPH
Weight gain of phase 1 (g)	132,21 $\pm$ 6,79 ^b	140,66 $\pm$ 9,92 ^{ab}	148,6 $\pm$ 8,41 ^a	129,18 $\pm$ 5,60 ^b
Weight gain of phase 2 (g)	39,38 $\pm$ 2,21 ^a	-35,80 $\pm$ 3,11 ^b	-37,47 $\pm$ 9,3 ^b	-31,45 $\pm$ 4,22 ^b
Diet intake of phase 1 (g)	23,2 $\pm$ 0,9 ^a	17,3 $\pm$ 0,8 ^b	17,3 $\pm$ 0,7 ^b	16 $\pm$ 0,4 ^b
Diet intake of phase 2 (g)	20,6 $\pm$ 0,9 ^a	9,19 $\pm$ 1,2 ^b	9,2 $\pm$ 1,2 ^b	10,8 $\pm$ 1,2 ^b
Energy efficiency of phase 1 (g/kcal)	7,0 $\pm$ 0,1 ^c	8,1 $\pm$ 0,2 ^a	8,3 $\pm$ 0,1 ^a	7,5 $\pm$ 0,1 ^b
Energy efficiency of phase 2 (g/kcal)	3,4 $\pm$ 0,1 ^a	-6,1 $\pm$ 1,2 ^b	-4 $\pm$ 1,2 ^b	-3,9 $\pm$ 0,7 ^b
Glucose (mg/dL)	123.55 $\pm$ 13.1 ^b	358.28 $\pm$ 50.9 ^a	456.14 $\pm$ 98.1 ^a	372.14 $\pm$ 80 ^a
TC (mg/dl)	82.87 $\pm$ 10 ^a	105.64 $\pm$ 39.9 ^a	129.32 $\pm$ 58.6 ^a	102.84 $\pm$ 39.4 ^a
HDL (mg/dL)	69.51 $\pm$ 12.6 ^a	55.03 $\pm$ 11.4 ^b	54.44 $\pm$ 6.2 ^b	50.57 $\pm$ 4.5 ^b
TAG (mg/dL)	141.4 $\pm$ 48.1 ^b	256.3 $\pm$ 113 ^{ab}	294.6 $\pm$ 106.3 ^a	331.1 $\pm$ 166.4 ^a
AST (U/l)	38.8 $\pm$ 9.6 ^a	47.1 $\pm$ 12.1 ^a	54.4 $\pm$ 17.6 ^a	41.9 $\pm$ 8.9 ^a
ALT (U/l)	28.3 $\pm$ 11.3 ^b	48 $\pm$ 13.1 ^{ab}	67.2 $\pm$ 26.9 ^a	50.5 $\pm$ 16 ^a
Albumin (g/L)	4.38 $\pm$ 0.36 ^a	4.42 $\pm$ 0.15 ^{ab}	4.23 $\pm$ 0.21 ^{ab}	4.33 $\pm$ 0.14 ^b
Total protein (g/L)	6.4 $\pm$ 0.5 ^a	6.3 $\pm$ 0.5 ^a	6.4 $\pm$ 0.5 ^a	6.2 $\pm$ 0.8 ^a
Insulin (ng/mL)	8.6 $\pm$ 2.7 ^a	3.3 $\pm$ 1.1 ^b	3.7 $\pm$ 1.4 ^b	5.8 $\pm$ 2.1 ^b

We do not rule out the possibility that the form or agent used to induce diabetes was responsible for the unexpected response to the type of protein used in the diet. However, it was certainly surprising to discover that the casein turned out to produce a less harmful response in the diabetic animals than did the whey proteins. It would be necessary to use genetically modified animals or test other types of induction of diabetes to evaluate these results more thoroughly.

4. CONCLUSION

It follows that, when diabetes mellitus is induced by this technique, the milk whey protein hydrolyzate was more effective in controlling body weight than the non-hydrolyzed protein or casein. However, this state appears to have been reached at the expense of the animal's health because ingestion of the WPH for a period of three weeks preceding the application of the antibiotic STZ did not prevent, nor significantly alleviate the hyperglycemia and appearance of acute liver damage caused by the application of both HF and STZ. In summing, as a result of the aggressive treatment used to induce DM, consumption of protein from whey, in its two forms, proved to be generally damaging compared to casein, as evidenced by the negligible mitigation effect and significant liver injury observed during the time span of the experiment. Considering the numerous health benefits attributed to the milk whey proteins, doubts are cast as to the appropriateness of streptozotocin for the experimental induction of diabetes.

5. ACKNOWLEDGMENTS

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ANEXOS



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CEUA/Unicamp**

CERTIFICADO

Certificamos que o projeto "Efeitos do consumo de proteínas do soro de leite intactas e hidrolisadas nos parâmetros de resistência à insulina induzida com dieta hiperlipídica associada a Estreptozotocina em ratos Wistar" (protocolo nº 2913-1), sob a responsabilidade de Prof. Dr. AIME AMAYA-FARFÁN / KELLY FERREIRA DOS SANTOS, está de acordo com os **Princípios Éticos na Experimentação Animal** adotados pela **Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL)** e com a legislação vigente, **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, e o **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**.

O projeto foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP - em 28 de novembro de 2012.

Campinas, 28 de novembro de 2012.

Prof. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva